

# Minor variant of rs 16827043 in the iron regulator hemojuvelin gene (HJV) contributes to hypertension

## The TAMRISK study

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### Abstract

It is known that iron overload may lead to an increased risk for many diseases. According to GWAS studies, iron regulatory protein HFE gene variant H63D (rs1799945) was associated with hypertension, an observation which we were able to confirm also in our TAMRISK cohort. Thus, it is possible that abnormalities in iron homeostasis may predispose to hypertension. This prompted us to study whether there is an association between hypertension and another iron overload-associated gene, hemojuvelin (HJV), which has 2 common polymorphic sites (rs 16827043, rs7536827).

The study included 336 hypertensive cases and 480 controls. All participants were 50-year-old Finnish men and women, and the data was collected from the Tampere adult population cardiovascular risk study (TAMRISK). Genotypes were determined using Competitive Allelic Specific PCR (KASP).

We found that the minor variant of the HJV polymorphic site rs16827043 (G-allele) is a statistically significant factor associated with hypertension among 50-year-old individuals compared with the AA genotype carriers (OR = 1.66, 95% CI: 1.06 – 2.60,  $P=0.03$ ). The risk was even higher when overweight subjects (BMI > 30) were excluded from the analyses. For the other polymorphic variant rs7536827, association with hypertension was found only among normal or slightly overweight A-allele carriers.

In conclusion, HJV genetic variants were associated with essential hypertension in Finnish subjects from the TAMRISK cohort. Previous studies together with the present one indicate that individuals with possible dysregulation of iron metabolism may have higher risk for hypertension than those with normal iron homeostasis.

**Abbreviations:** BMI = body mass index, CI = confidence interval, HFE = histocompatibility complex class I-like transmembrane protein (hemochromatosis protein), HJV = hemojuvelin (previously HFE2), PCR = polymerase chain reaction, PHE = periodic health examination.

**Keywords:** genetic variants, HJV, hypertension, iron

## 1. Introduction

Iron metabolism has been studied extensively to understand how the body maintains iron homeostasis. It is now known that hepcidin plays an important role on intestinal iron absorption and iron recycling in macrophages. Hepcidin functions by decreasing the amount of iron released from macrophages or

absorbed from intestine. It is also known that hepcidin deficiency may lead to severe iron overload in multiple organs.<sup>[1–4]</sup> Hepcidin synthesis is complexly regulated by different proteins and pathways.<sup>[5]</sup> Two important transmembrane proteins, HFE (histocompatibility complex class I-like transmembrane protein) and HJV (hemojuvelin), are key modulators of hepcidin expression. HJV acts as a bone-morphogenetic protein (BMP) co-repressor, driving hepcidin transcription via the BMP-SMAD signaling cascade.<sup>[6]</sup> Using a mouse model, Kent et al<sup>[7]</sup> showed that HFE and HJV operate in the same pathway for regulation of hepcidin expression and iron metabolism. Recently, Wu et al<sup>[8]</sup> showed that HJV is the key regulator of hepcidin and that HFE acts in an HJV-dependent manner. Some mutations in these 2 proteins have been associated with severe iron overload in patients with hereditary haemochromatosis.<sup>[9,10]</sup>

Dysregulation in iron homeostasis may also lead to mild iron overload, which has not been generally taken into account. Two previous GWAS studies<sup>[11,12]</sup> have found an association between HFE (H63D) genetic variant and hypertension. We were able to replicate this association in the TAMRISK cohort and showed that carriers of the mutation had higher risk for hypertension than those without this mutation.<sup>[13]</sup>

Mutations in the HJV gene that lead to severe iron overload are rare, although over 40 mutations of HJV have been recognized.<sup>[14]</sup> Therefore, we analyzed 2 relatively frequent variants of this gene that have been shown to mediate dysfunctional iron

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regulation.<sup>[15]</sup> In the present study, we wanted to examine a possible association between these HJV genetic variants and hypertension in the Finnish TAMRISK cohort.

## 2. Materials and methods

### 2.1. Study population

The Tampere adult population cardiovascular risk study (TAMRISK) is a prospective, longitudinal population-based health survey study in Tampere, a city in southern Finland with a population of 210 000. The data for the TAMRISK study was collected from the periodic health examinations (PHE) done for 50-year-old men and women living in Tampere. The PHE included one 60-minute session with a public health nurse at the center's health examination unit as previously described.<sup>[13,16]</sup> TAMRISK data includes information of risk factors for hypertension: blood pressure, weight, family history of cardiovascular diseases, lipid values and smoking, diabetes, and exercise habits. Physical activity was defined as times of exercise/week (enhanced breathing and sweating). Current and previous diseases were identified based on self-report of diagnosis by a physician, including hypertension. Cases in this study were the subjects who had hypertension and/or CAD at the age of 50 years as diagnosed by a physician by normal healthcare procedures. For most patients, physicians diagnose hypertension when blood pressure readings are consistently 140/90 mm Hg or above. For each case, at least 1 normotensive control with the same sex and similar smoking habits were chosen from a PHE cohort (n = 6000). Smoking status was evaluated based on self-reporting.

Using the patient's national identity code, data on hospitalizations including ICD-10 codes for discharge diagnoses were obtained from the Finnish National Hospital Discharge Registry (HILMO) maintained by the National Institute of Health and Welfare. Prevalence of ischemic heart diseases (I20-I25) were followed up from 2005 to 2014 until the subjects were on the average 60 years old.

Buccal swabs for DNA extraction and a permissions form to use PHE data were collected by mail separately of the physical examination. The DNA samples were collected during years 2006–2010. Informed consent was obtained from all partic-

ipants. The Ethics Committees of the Tampere University Hospital and the City of Tampere approved the study.

### 2.2. Genotyping

DNA was extracted from buccal swabs using a commercial kit (Qiagen Inc., Valencia, CA). Genotyping was performed using KASP (Competitive Allelic Specific Amplification) genotyping services at KBioscience Institute, UK. Details of this method can be obtained from <https://www.lgcgroup.com/genotyping/>.

### 2.3. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 23, and Hardy–Weinberg equilibrium of the genotypes was calculated using OEGE (online encyclopedia calculator for genetic epidemiology studies).<sup>[17]</sup> T-test and 1-way ANOVA for continuous variables (clinical characteristics) and chi-square test for categorical variables (hypertension, coronary artery disease) were applied for the comparison of HJV genotype groups. Associations of the genotyped HJV gene variants with hypertension/coronary artery disease with risk factors (BMI, glucose, cholesterol and gender) were analyzed using logistic regression analysis. *P*-values less than 0.05 were considered significant.

## 3. Results

Clinical characteristics of the study population at the age of 50 years are presented in Table 1. Briefly, the case group comprised 336 hypertensive cases and control group 480 normotensive subjects with the same sex distribution and similar smoking habits. A total of 78 subjects were found to have coronary artery disease when followed up to 60 years of age.

Genotyping was successful in 808 subjects for the HJV rs16827043 (A > G) and in 795 subjects for the rs7536827 (A > T). The measured genotype frequencies were not significantly different from the expectations of Hardy–Weinberg equilibrium ( $\chi^2=0.22$  for rs16827043 and  $\chi^2=0.09$  for rs7536827). The clinical characteristics according to different genotypes are shown in Tables 2 and 3.

**Table 1**  
Clinical characteristics of cases and controls of the study population and subpopulation with BMI < 30.

	Study population			Subpopulation with BMI < 30		
	Cases (n=336)	Controls (n=480)	<i>P</i>	(Cases n=239)	Controls (n=399)	<i>P</i>
Age, y	50±0	50±0		50±0	50±0	
BMI, kg/m <sup>2</sup>	28.8±5.1	25.5±3.7	<0.001	25.9±2.8	24.8±2.7	<0.001
Hemoglobin	147.0±13.4	145.4±13.2	0.165	146.3±13.0	144.8±13.1	0.240
Cholesterol, mmol/L	5.38±0.99	5.37±0.88	0.887	5.42±1.02	5.37±0.88	0.510
LDL cholesterol, mmol/L	3.16±0.88	3.17±0.82	0.838	3.15±0.92	3.16±0.83	0.921
Glucose, mmol/L	5.17±1.29	4.86±0.53	<0.001	5.11±1.45	4.85±0.53	0.001
Systolic blood pressure, mm Hg	142.7±16.6	129.3±14.8	<0.000	142.7±17.0	128.4±14.3	<0.000
Diastolic blood pressure, mm Hg	92.8±8.8	84.4±9.1	<0.000	92.7±8.9	83.8±8.7	<0.000
Hypertension %	100	0		100	0	
Diabetes %	12.8	0	<0.000	9.6	0	<0.000
Myocardial infarction %	3.6	0	<0.000	3.2	0	0.001
Exercise, at least twice a week, %	62.8	57.2	0.135	62.8	55.5	0.102
Family history of hypertension %	71.6	42.5	<0.000	74.7	41.5	<0.000
Gender, male, %	58.4	56.8	0.319	58.3	64.1	0.165

Data is presented as mean ± SD

BMI = body mass index, LDL = low density lipoprotein, SD = standard deviation.

**Table 2****Clinical characteristics of the study population stratified according to HJV rs 16827043 genotypes.**

HJV rs16827043	AA	AG	GG	<i>P</i> AA vs AG vs GG	<i>P</i> AA vs (AG + GG)
n at 50	697	108	3		
Hypertension %	41.3	51.0	100	<b>0.024</b>	<b>0.041</b>
Coronary artery disease, %, n=78	9.9	8.3	0	0.745	0.729
Systolic blood pressure, mm Hg	134.7 ± 16.8	137.7 ± 16.7	136.7 ± 13.3	0.237	0.095
Diastolic blood pressure, mm Hg	87.7 ± 9.8	89.6 ± 9.7	98.9 ± 7.2	<b>0.035</b>	<b>0.033</b>
Body mass index, kg/m <sup>2</sup>	26.9 ± 4.7	27.1 ± 4.2	25.5 ± 2.3	0.784	0.746
Cholesterol, mmol/L	5.35 ± 0.99	5.55 ± 0.90	5.53 ± 0.97	0.151	0.052
Glucose, mmol/L	5.03 ± 1.20	5.02 ± 0.68	4.83 ± 0.25	0.953	0.898

HJV = hemojuvelin (previously HFE2).

*P* values from the chi-square test for categorical variables and 1 way ANOVA or T-test for continuous variables.*P* values < 0.05 are in bold.

For HJV polymorphism rs16827043, there were only 3 individuals who were homozygous for the GG genotype and they were combined to the GA genotype group. At the age of 50 years, 58 of 111 G-allele carriers (52.2%) had diagnosed hypertension compared to 287 of 697 (41.3%) of those homozygous for the wild type (AA), respectively ( $P=0.041$ ). Also, diastolic blood pressure readings were significantly higher among G-allele carriers (Table 2). When the risk for hypertension was analyzed by logistic regression using HJV variants, BMI, glucose, cholesterol, and gender as explainable variables, OR for HJV G-allele carriers was 1.66 ( $P=0.03$ , 95% CI: 1.06–2.60), for BMI 1.19 ( $P<0.001$ , 95% CI: 1.15–1.24), for glucose 1.49 ( $P<0.001$ , 95% CI: 1.16–1.92), for cholesterol 0.99 ( $P=0.96$ , 95% CI: 0.84–1.18), and for gender 1.50 ( $P=0.02$ , 95% CI: 1.07–2.09) compared with AA genotype. In order to exclude the strong effect of BMI on hypertension, we also analyzed a subpopulation of the study participants with normal or only slightly elevated BMI. When overweight participants (BMI > 30) were excluded from the analyses, the risk for hypertension among G-allele carriers remained significant (OR=1.80, 95% CI 1.09–2.98,  $P=0.02$ ). Adjusted and unadjusted results are in Table 4. No statistically significant association with coronary artery disease was found (Table 2).

For the other polymorphic site (rs7536927, T>A), no association between genotypes or combined alleles and hypertension was found in the whole study population ( $P=0.29$  for genotypes and  $P=0.24$  for A-allele carriers). However, when subjects were stratified according to weight, carriers of the A-allele whose BMI was less than 30 had more often hypertension compared with those homozygous for the wild type

(TT) (OR=1.56,  $P=0.04$ , 95% CI 1.01–2.39) (Table 4.) As with the rs7536827, no association with coronary artery disease was found (Table 2).

#### 4. Discussion

The results of the present study suggest that genetic variation in the HJV gene is significantly associated with hypertension in a 50-year-old Finnish population. Previous studies of the HJV and HFE genetic polymorphisms have concentrated mainly on hemochromatosis. Our results and those of others indicate that disturbances in iron metabolism may also increase the risk for hypertension.<sup>[18,19]</sup> We found no impact of the 2 studied HJV polymorphisms on coronary artery disease.

In this paper, we show that the minor allele G of the HJV variant rs16827043 was associated with hypertension. In addition, the association between the minor allele and hypertension was even stronger among normal- or only slightly overweight subjects. For the other HJV polymorphic site rs7536827, no statistically significant association with hypertension was found. However, when T allele carriers were combined and obese subjects were excluded, also this genetic variation associated with hypertension. Although the mechanism is not known, our result together with previous ones provides a further link between iron metabolism and hypertension.<sup>[11–13,20]</sup>

Mild iron overload is one possible explanation for higher blood pressure of carriers of the HJV minor variants, as has been suggested for H63D.<sup>[13]</sup> Both of these membrane receptors are involved in pathways leading to hepcidin transcription. A recent

**Table 3****Clinical characteristics of the study population stratified according to HJV rs 7536827 genotypes.**

HJV rs7536827	AA	AT	TT	<i>P</i> AA vs AT vs TT	<i>P</i> AA vs (AT + TT)	<i>P</i> TT vs (AA + AT)
n at 50	186	397	212			
Hypertension %	39.2	45.5	40.9	0.293	0.602	0.241
Coronary artery disease, %, n=78	8.1	10.4	9.4	0.680	0.161	1.000
Systolic blood pressure, mm Hg	134.9 ± 16.3	135.5 ± 16.6	134.4 ± 17.7	0.738	0.898	0.475
Diastolic blood pressure, mm Hg	87.6 ± 10.2	88.5 ± 9.7	87.4 ± 9.6	0.362	0.529	0.302
BMI, kg/m <sup>2</sup>	26.6 ± 4.8	27.1 ± 4.6	26.9 ± 4.5	0.406	0.250	0.767
Cholesterol, mmol/L	5.37 ± 0.95	5.34 ± 1.05	5.46 ± 0.89	0.340	0.846	0.151
Glucose, mmol/L	5.03 ± 0.91	5.01 ± 1.19	5.07 ± 1.24	0.841	0.949	0.567

BMI = body mass index.

*P* values from the chi-square test for categorical variables and 1-way ANOVA or T-test for continuous variables.*P* values < 0.05 are in bold.

**Table 4****Unadjusted and adjusted OR results obtained from logistic regression analysis for hypertension.**

	Univariate model <sup>*</sup>		Multivariate model <sup>†</sup>	
	OR (95% CI)	P	OR (95% CI)	P
All participants				
rs16827043, G-allele vs AA	1.54 (1.02–2.32)	0.041	1.66 (1.06–2.60)	0.028
rs7536827, A-allele vs TT	1.26 (0.88–1.69)	0.247	1.36 (0.94–1.98)	0.103
<b>BMI &lt; 30 kg/m<sup>2</sup></b>				
rs16827043, G-allele vs AA	1.70 (1.05–2.75)	0.032	1.80 (1.09–2.98)	0.022
rs7536827, A-allele vs TT	1.48 (0.99–2.22)	0.056	1.56 (1.01–2.39)	0.044

BMI = body mass index, CI = confidence interval, OR = odds ratio.

<sup>\*</sup> Univariate model with HJV SNP alone<sup>†</sup> Multivariate<sup>2</sup> model with HJV SNP, BMI, glucose, cholesterol, and gender as explainable variables

study has reported that HJV functions as enhancer for iron signaling to hepcidin. Since hepcidin is the main iron regulatory hormone, disturbances in pathways affecting hepcidin expression may block its function as a feedback inhibitor of iron absorption. However, only complete loss of hepcidin in humans is responsible for rare yet severe forms of massive body iron overload.<sup>[21]</sup>

In Finland, tests for assessing body iron levels (serum ferritin and transferrin saturation) are not routinely measured and therefore a limitation of the TAMRISK study population is the lack of these saturation markers. However, it has previously been published that men with essential hypertension had greater iron stores than normotensive controls.<sup>[20]</sup>

It is known that prevalence of hypertension is higher in obese than in lean populations and there is nearly linear relationship between BMI and blood pressure.<sup>[22]</sup> It has previously been shown that there is a gene-environmental association between hypertension genes and BMI. Thus, obesity will override the genetic effect.<sup>[13,23]</sup> The association of HJV and hypertension was similar or even stronger for normal weight and slightly overweight subjects than in obese subjects, confirming the role of HJV.

Although the mechanism is not yet known, our results suggest that the minor allele G of the HJV variant (rs16827043) is associated with higher risk for hypertension at the age of 50 years, compared with the AA-genotype carriers. In addition, we found that among normal or slightly overweight individuals, also T-allele of the HJV rs7536827 increases the risk for hypertension. It is therefore possible that dysregulation in iron metabolism is a significant factor behind hypertension. Abnormalities in iron homeostasis and relationship between iron metabolism and hypertension warrant further studies.

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## References

- Crownover B, Covey C. Hereditary hemochromatosis. *Am Fam Physician* 2013;87:183–90.
- Means RT Jr. Hepcidin and iron regulation in health and disease. *Am J Med Sci* 2013;345:57–60.
- Loreal O, Haziza-Pigeon C, Troadec M, et al. Hepcidin in iron metabolism. *Curr Protein Peptide Sci* 2005;6:279–91.
- Nicolas G, Chauvet C, Viatte L, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia and inflammation. *J Clin Invest* 2002;110:1037–44.
- Silva B, Faustino P. An overview of molecular basis of iron metabolism regulation and the associated pathologies. *Biochimica et Biophysica Acta* 2015;1852:1347–59.
- Babitt JL, Huang FW, Wrighting DM, et al. Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. *Nat Genet* 2006;38:531–9.
- Kent P, Wilkinson N, Constante M, et al. Hfe and Hvj exhibit overlapping functions for iron signaling to hepcidin. *J Mol Med* 2015;93:489–98.
- Wu Q, Wang H, An P, et al. HJV and HFE play distinct roles in regulating hepcidin. *Antioxid Redox Signal* 2015;22:1325–36.
- Feder J, Gnirke A, Thomas W, et al. A novel MCH class I-like gene is mutated in patients with hereditary haemochromatosis. *Nature Genet* 1996;13:399–408.
- Papanikolaou G, Samuels ME, Ludwig EH, et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. *Nat Genet* 2004;36:77–82.
- Ehret GB, Munroe PB, Rice KM, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 2011;478:103–9.
- Lu X, Wang L, Lin X, et al. Genome-wide association study in Chinese identifies novel loci for blood pressure and hypertension. *Hum Mol Genet* 2015;24:865–74.
- Määttä KM, Nikkari ST, Kunnas TA. Genetic variant coding for iron regulatory protein HFE contributes to hypertension, the TAMRISK study. *Medicine (Baltimore)* 2015;94:e464.
- Core A, Canali S, Babitt J. Hemojuvelin and bone morphogenetic protein (BMP) signaling in iron homeostasis. *Front Pharmacol* 2014;5:104.
- Milet J, Dehais V, Bourgain C, et al. Common variants in the BMP2, BMP4 and HJV genes of the hepcidin regulation pathway modulate HFE hemochromatosis penetrance. *Am J Hum Genet* 2007;81:799–807.
- Määttä KM, Nikkari ST, Lähtelä KH, et al. A functional variant in the serine-threonine kinase coding gene is associated with hypertension: a case-control study in a Finnish population, the Tampere adult population cardiovascular risk study. *J Hypertension* 2013;31:516–20.
- Rodrigues S, Gaunt TR, Day INM. Hardy–Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am J Epidemiol* 2009;169:505–14.
- Natekar A, Olds RL, Lau MW, et al. Elevated blood pressure: our family's fault? The genetics of essential hypertension. *World J Cardiol* 2014;6:327–37.
- Valenti L, Maloberti A, Signorini S, et al. Iron stores, hepcidin, and aortic stiffness in individuals with hypertension. *Plos One* 2015;10:e0134635.
- Piperno A, Trombini P, Gelosa M, et al. Increased serum ferritin is common in men with essential hypertension. *J Hypertens* 2002;20:1513–8.
- Pietrangolo A. Genetics, genetic testing, and management of hemochromatosis: 15 years since hepcidin. *Gastroenterology* 2015;149:1240–51.
- Weinberger MH, Fineberg NS, Fineberg SE, et al. Salt sensitivity, pulse pressure, and death in normal and hypertensive humans. *Hypertension* 2001;37:429–32.
- Marteau J-B, Sass C, Pfister M, et al. The Leu554Phe polymorphism in the E-selectin gene is associated with blood pressure in overweight people. *J Hypertens* 2004;22:305–11.